CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
2,4-D
[This document includes data on some salts and esters, with unique Tolerance #s]

Chemical Code # 000636 Tolerance # 00142
SB 950 # 176

September 26, 1986
Revised 11/4/88, 5/18/89, 8/01/91, 8/16/93, 7/19/94, 12/12/95, 1/16/96, 2/24/00, and 8/17/06

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect*
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotoxicity:	Not required at this time**

* Liver tumors appeared to be elevated at and above the "MTD" in male mice, based on small numbers of adenomas in an interim sacrifice group in an aborted study. Reducing dose levels only slightly in a replacement study eliminated liver tumor effects (see below).
** There are no avian neurotoxicity studies on file, but there are acceptable rat neurotoxicity studies (one acute and one chronic).

Toxicology one-liners are attached.

All record numbers for the above study types through 204131 (Document No. 142-0226) were examined. This includes all relevant studies indexed by DPR as of 8/15/06.

In the 1-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: t20060817.wpd
Revised by Aldous, 8/17/06.

Related Active Ingredients: Currently all data on active ingredient relevant to this Summary of Toxicology Data are submitted under the tolerance number for the free acid. There are several registered salts and esters of 2,4-D. Some older studies have been submitted under tolerance numbers 50721 and 50730 (see below), and these are included in this Summary. No new reports of studies relevant to this Summary were found under tolerance numbers other than #142 upon searching for study reports for all major salts and esters of 2,4-D with active registrations. Aldous, 10/29/99.

2,4-D (SB #176) is the "Lead Chemical" for the series of 2,4-D salts and esters (SB # 177 to SB # 197). This toxicology summary includes any existing 1-liners for the following 2,4-D salts and esters, as well as the free acid:

2,4-D Dimethylamine (DMA) salt: Chemical Code # 000806, Tolerance # 50721, SB 950 # 178

2,4-D, 2-Ethylhexyl Ester: Chemical Code # 001622, Tolerance # 50730, SB 950 # 189

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

In the 1-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

Note: these pages contain summaries only. Individual worksheets may identify additional effects.

COMBINED, RAT

The chronic NOEL is 1 mg/kg/day, based on increased pigmentation in tubular epithelium of kidneys of both sexes in the 1986 Hazleton study and in males in the 1995 Dow study (Record No. 156537), also mineralization of kidney pelvic epithelium in the latter study in females. The high dose led to excessive body weight decrements, particularly in females. Also there was unusual histopathology at that dose level (increases or decreases compared to normal), suggesting that an MTD had been exceeded at 150 mg/kg/day. "Possible adverse effects" relate to the severities of several high dose effects: no oncogenicity was indicated. Aldous, 2/24/00, 8/17/06.

****142-175 156537** Jeffries, T. K, B. L. Yano, J. R. Ormand, and J. E. Battjes, "2,4-Dichlorophenoxyacetic acid: Chronic toxicity/oncogenicity study in Fischer 344 rats", The Dow Chemical Company, Midland, MI, 3/28/95. Laboratory Project Study No. K-002372-064. This was a standard combined study, with 50 F344 rats/sex/group at 0, 5, 75, or 150 mg/kg/day of 2,4-D (96.45%). Exposure was by diet, with concentrations adjusted according to group body weight and food consumption patterns. Additional groups of 15 rats/sex/group were used for a 1-year study. Of these, 5/sex/group were allocated to a neuropathology group, and 10 to a standard interim sacrifice group. FOB evaluations were performed at about 3, 6, 9, and 12 months into the study on all neuropathology rats and on a pre-selected 5/sex/group of the interim sacrifice rats. The FOB report is a separate document to be reviewed separately. Multiple sections of brains were examined microscopically, since an earlier study (Record No. 047270, the 1986 Hazleton combined F-344 rat study) had indicated a possible brain tumor effect. NOEL = 1 mg/kg/day (based on increase in incidence and/or degree of pigmented tubular cell epithelium over the range of 5 to 75 mg/kg/day, most consistently in males). Many findings at 75 mg/kg/day were limited to females, including reduced body weight, reduced blood parameters (RBC count, Hb, HCT, platelet count), reduced ovarian weights at 2-year sacrifice, increased thyroid weights, increased hepatocyte size and altered tinctorial properties, and alveolar histiocytosis and increased chronic or subchronic inflammation in the lungs. Dose-related degeneration of the descending portion of the kidney proximal tubule was observed to a slight degree in 75 mg/kg/day males and females at 1 year, but this response did not persist in the 2-year study. Clinical chemistry findings at 75 to 150 mg/kg/day included: slightly elevated alanine aminotransferase activities in males, slightly increased alkaline phosphatase and aspartate aminotransferase activities in both sexes, slightly reduced glucose and globulin levels in females, and reduced cholesterol and elevated creatinine in both sexes. Dose-related reductions in thyroid hormone (T_4) were found in both sexes at 75 to 150 mg/kg/day, with the strongest reductions in females. Study found no oncogenicity (supersedes Record No. 047270 in this respect). Study indicates a "possible adverse effect", based upon several changes of remarkable incidence or degree at 150 mg/kg/day and occasionally at 75 mg/kg/day [ocular changes (cataracts and retinal degeneration), heart degeneration, and lung inflammation and histiocytosis, substantial drop in circulating thyroxin, and proximal tubular degeneration at 1-yr sacrifice]. Supplement in 142-176 156538 provided histopathology data on brains of intermediate group male rats, which data are incorporated into this review. These results do not indicate any treatment effect on brain tumor incidence, thus there is no overall indication of

oncogenicity in rats. The high dose led to excessive body weight decrements, particularly in females; also there was unusual histopathology at that dose level (increases or decreases compared to normal), suggesting that an MTD had been exceeded at 150 mg/kg/day. Aldous, 12/31/99. Re-examined by Aldous with worksheet on 8/17/06 to reduce NOEL from 5 mg/kg/day to 1 mg/kg/day, consistent with table values from the original review.

142-157 132112 one year interim report of Record No. 156537, above. Interim report was examined by Aldous on 12/06/95.

****142-105** to **-107 047270-047272**, "Combined toxicity and oncogenicity study in rats: 2,4-Dichlorophenoxyacetic acid" [Final Report]. Hazleton, (Vienna, VA), 5/29/86. 2,4-D, 97.5% purity, 0, 1, 5, 15, or 45 mg/kg/day, fed to Fischer 344 rats, 60/sex/group, for 104 weeks. NOEL = 1 mg/kg/day (kidney tubular cell pigmentation at 5 mg/kg/day). **Possible adverse effect**: [low NOEL for kidney effects, also astrocytomas were increased in males (incidence of 1, 0, 0, 2, and 6 for increasing dose groups)]. Record No. 047272 contains pathologist's interpretation of tumor data, which review suggests that increased tumors in high dose group were incidental, since several features commonly observed in treatment-caused astrocytomas were not observed in this study. ACCEPTABLE. (J. Gee, 9/25/86 and C. Aldous, 8/1/88).
NOTE: EPA requested an additional oncogenicity study, the final report of which is Record No. 156537.

EPA concluded in Federal Register 53 (56), 9590-9594, dated 3/23/88: that the Fisher-Exact test was negative, and the Cochran-Armitage trend test was marginally positive for increased incidence of astrocytomas in males, thus "neither evaluation found strong statistical evidence of oncogenicity in the rat".

142-111:[no record #] Rebuttal to 105-107:047270-047272. Cites pathology consultant's comments in 107:047272, above; gives an example of uneven distribution of gliomas in rat brains from two control groups in another study; notes that there were no increased tumors in a recent 2,4-D study with B6C3F1 mice. No new data were presented in this rebuttal with respect to the cited 2,4-D rat study. This submission was discussed in a supplemental worksheet to 105-107:047270-047272 by C. Aldous, 8/1/88.

142-088 028383 Interim report to 105:047270.

142-157 132112 Jeffries, T. K, B.L. Yano, and J. R. Ormand, "2,4-Dichlorophenoxyacetic acid (2,4-D): Chronic toxicity/oncogenicity study in Fischer 344 rats - one year interim report", The Dow Chemical Co., Midland MI, 6/23/93, Laboratory Project Study ID# K-002372-064I. Ten rats/sex/group were assigned to the 1-yr interim sacrifice portion of the combined study. Rats received 0, 5, 75, or 150 mg/kg/day 2,4-D (96.4%). Ten/sex/group received microscopic examinations in at least controls and high dose groups. Intermediate groups were examined where indicated. NOEL = 5 mg/kg/day [noteworthy findings at the LEL included slight kidney proximal tubule degeneration; alveolar histiocytosis in lungs of females; hepatocyte enlargement with some altered staining characteristics in females; several clinical chemistry changes,

particularly marked decrements in thyroxin in both sexes (thyroid weights were elevated in high dose males and females, and in 75 mg/kg/day females, however thyroid histopathology was limited to very slight decrease in thyroglobulin in females); hematology changes in females (decreased RBC count, decreased HCT); small reductions in body weights in females]. Bilateral testicular tubular atrophy was observed in 150 mg/kg/day males. Study is valid as an interim report, but does not independently fill a data requirement. A "possible adverse effect" is indicated, largely due to the substantial drop in circulating thyroxin levels in both sexes, and to retinal degeneration in 150 mg/kg/day females. Aldous, 12/06/95.

142-120 071907 "Acute, pharmacokinetic, and subchronic toxicological studies of 2,4-Dichlorophenoxyacetic acid". Report in Fundam. Appl. Toxicol. 9, 423-435 by Gorzinski et al. This report was submitted primarily in justification for dosage selection for primary study 105:047270. This published report did not require CDFA written review, since the study had already been accepted by CDFA; however the review contents were discussed in CDFA rebuttal response of 5/17/89, by C. Aldous.
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SUBCHRONIC RAT

****142-137 098298** "Subchronic Toxicity Study in Rats with 2,4-Dichlorophenoxyacetic Acid", (Gene E. Schulze, Ph.D., D.A.B.T., Hazleton Laboratories America, Rockville, MD., August 7, 1991). 2,4-dichlorophenoxyacetic acid, 96.1% purity, was administered in the diet for 13 weeks at 0, 1, 15, 100, and 300 mg/kg/day to 10 Fischer 344 rats per sex per group. NOEL = 15 mg/kg/day [based primarily on dose-related decrements in body weights and in food consumption (both sexes); marked decrement in thyroid hormones (especially T4, both sexes); dose-related increases in cataracts (females); hypertrophy of zona glomerulosa of adrenal cortex (both sexes, more prominent in females)]. The study indicates "**possible adverse effects**", due to cataracts, retinal degeneration, thyroid hormone decrements, adrenal cortex effects; and secondarily to testicular atrophy, which was limited to 300 mg/kg/day males. The high dose exceeds the "MTD", based on large body weight and food consumption decrements, and on the wide range of toxic responses at that dosage. Study is **acceptable**. H. Green and C. Aldous, 3/25/93.

50721-039 097502 Schulze, G. E., "Subchronic toxicity study in rats with the dimethylamine salt of 2,4-dichlorophenoxyacetic acid", Hazleton Laboratories America, Inc., April 9, 1991. This study was undertaken to establish dose levels for an eventual 2-year rat feeding study. Ten Fischer-344 rats/sex/group were given 0, 1.2, 18.1, 120, or 361 mg/kg/day of test article (expressed as 2,4-D-DMA) in diet for at least 13 weeks. NOEL = 18.1 mg/kg/day. Body weight and food consumption were reduced substantially in 361 mg/kg/day rats, and biologically significantly also in 120 mg/kg/day females. Various hematology changes were noted, especially decreases in counts of various blood cell types, generally in 120 and 361 mg/kg/day groups. Thyroid follicular cell hypertrophy was noted in some 361 mg/kg/day males and females, and thyroid hormone levels were generally reduced in 120 and 361 mg/kg/day males and females. Retinal degeneration and cataract formation in 361 mg/kg/day females, testicular

atrophy in 361 mg/kg/day males, hypertrophy of the zona glomerulosa of the adrenal cortex, loss of brush border cells in proximal tubular cells of kidney in both sexes, centrilobular hepatocellular hypertrophy in females, and hypoplasia of spleen (females) and bone marrow (both sexes) were noted in histopathology. No "possible adverse effects" are noted, however clearly the appropriate high dose for the chronic study would be well above 18 mg/kg/day, and below 361 mg/kg/day (which appears to exceed the MTD). No Medical Toxicology Branch review is needed for SB-950 purposes. Aldous, 6/25/91. [Note: results of this study were compared with results of study 098298, above, in the discussion of the 1993 worksheet for that study].

Comparison of Subchronic Rat Studies:

This review compares the key findings in the six rat subchronic studies received for 2,4-D and its salts and esters as of 7/24/06. These are (beginning with DPR Document No. and Record No. for each): (1) 142-088 028523 Gorzinski, S. J. *et al.*, "Purified 2,4-dichlorophenoxyacetic acid (2,4-D): results of a 13-week subchronic dietary toxicity study in the CDF Fischer 344 rat," The Dow Chemical Company, Midland, MI, 1981. (2) 142-088 028524 Gorzinski, S. J. *et al.*, "Technical grade 2,4-dichlorophenoxyacetic acid (2,4-D): results of a 13-week subchronic dietary toxicity study in the CDF Fischer 344 rat," The Dow Chemical Company, Midland, MI, 1981. (3) 142-088 028526 Serota, D. G., "Subchronic toxicity study in rats: 2,4-dichlorophenoxyacetic acid (2,4-D)," Hazleton Laboratories America, Inc., Vienna, VA, 9/12/83, Project No. 2184-102. (4) 142-0136 089479 Schulze, G. E., "Subchronic Oral Toxicity Study in Rats with 2,4-dichlorophenoxyacetic acid-2-ethylhexyl ester," Hazleton Laboratories America Inc., Vienna, VA, 04/01/1991. HLA Study No. 2184-112. (5) 142-0137 098298 Schulze, G. E., "Subchronic Toxicity Study in Rats with 2,4-dichlorophenoxyacetic Acid," Hazleton Laboratories America, Inc. Rockville, MD, 08/01/1991. HLA Study No. 2184-116. (6) 50721-0039 097502 Schulze, G. E., "Subchronic Toxicity Study in Rats with the dimethylamine salt of 2,4-dichlorophenoxyacetic Acid," Hazleton Laboratories America, Inc. Rockville, MD, April 9, 1991. HLA Study No. 2184-113. **Results and discussion**: Data support a NOEL based on adaptive, reversible change, and an NOAEL based on apparently irreversible change. NOEL = 1 mg/kg/day, based on kidney tubular cytoplasmic vacuolation and/or tinctorial change incidence or degree, which was significantly elevated at 5 mg/kg/day and above in the Serota (1983) study. None of the other 5 rat subchronic studies available confirmed this low NOEL. Relative kidney weights at 15 mg/kg/day were statistically significantly reduced in males in 2/6 studies. Degree and/or incidence of cytoplasmic vacuolation was slightly (non-significantly) elevated in 15 mg/kg/day females in the 1981 Gorzinski *et al.* study [testing purified 2,4-D], consistent with a trend toward significantly elevated response at 60 mg/kg/day and above in that study and in another study by Gorzinski *et al.* (1981) [testing technical 2,4-D]. A subchronic NOAEL of 15 mg/kg/day for more persistent kidney change is based in part on tubular brush border loss in one 100 mg/kg/day female, and in several rats/sex in both sexes at 300 mg/kg/day and above. Common findings at exceedingly high dose levels (300 to 452 mg/kg/day) included severe body weight decrements, with disproportionate decrements in organ weights for thymus, testes, ovaries and pituitaries (females); and general increases in liver and thyroid weights. Thyroid follicular cell hypertrophy

was commonly observed at those dose levels, along with marked reductions in circulating thyroxin (T_4) and to a lesser extent, T_3, both at 100 mg/kg/day and above. Adrenal cortical hypertrophy of the zona glomerulosa was observed at 100 and 300 mg/kg/day (particularly in females). Cataracts and retinal degeneration were dose-related in females at 100 mg/kg/day and above. At the highest dose levels there were reduced RBC parameters; some clinical chemistry changes; liver hepatocellular hypertrophy; atrophy in thymus, spleen, and testes; and other changes. See Discussion section in the worksheet entitled "Subchronic rat NOEL evaluation." Aldous, 8/8/06.

CHRONIC, DOG

****142-151 127497** Dalgard, D. W., "52-Week Dietary Toxicity Study with 2,4-D in Dogs", Hazleton Washington, Inc. Report #HWA 2184-124, 11/29/93. Technical grade 2,4-D, reported purity 96.7%, was given in diet for 1 year to 5 beagles per sex per group at 0, 1, 5, or 10/7.5 mg/kg/day (high dose was reduced from 10 to 7.5 mg/kg/day after week 8 due to poor body weight gain). NOEL = 1 mg/kg/day [perivascular chronic active inflammation in liver (both sexes), pigment in liver sinusoidal lining cells (females), and pigment in tubular epithelium of kidney (both sexes)]. These histopathology changes were associated with clinical chemistry effects (increased blood levels/activities of BUN, creatinine, alanine aminotransferase, and cholesterol; decreased blood glucose levels). **Acceptable**, with a "**possible adverse effect**" (due to comparatively low NOEL for liver and kidney effects). H. Green and C. Aldous, 7/19/94.

142-133 095868 Schulze, G. E., "Subchronic toxicity study in dogs with 2,4-Dichlorophenoxyacetic acid", Hazleton Laboratories America, Inc., Vienna, VA HLA Study No. 2184-115; 12/14/90. Study was done to establish dose levels for a chronic study, for which dose levels were to be approved by EPA. Dose levels in this subchronic study were 0., 0.3, 1, 3, and 10 mg/kg/day by gelatin capsule, administered to 5 beagles/sex/dose. Body weights appeared appreciably decreased in 10 mg/kg/day males and females, in association with reduced food consumption. Characteristic clinical signs included soft, mucoid feces at 3-10 mg/kg/day in males and at 1-10 mg/kg/day in females; sometimes also sanguineous in 3-10 mg/kg/day females and in 10 mg/kg/day males. RBC parameters were depressed significantly in 10 mg/kg/day males. At 10 mg/kg/day, 3/10 dogs were sacrificed moribund. Thymic depletion was seen in two of them. Microscopic findings in 10 mg/kg/day and sometimes in 3 mg/kg/day dogs included testicular hypospermatogenesis and alterations in kidney proximal convoluted tubules. Proposed high dose level for the chronic study was 3 mg/kg/day, which seems defensible from the abstract summary. No separate Medical Toxicology Branch review is required at this time. Aldous, 7/1/91.

50721-049 112025 Proposed protocol for Record No. 127497, above.

142-111 055303 "Chronic Toxicity of 2,4-Dichlorophenoxyacetic Acid in Rats and Dogs." (Toxicol. Appl. Pharmacol. 20:122-129, 1971). Technical 2,4-D was given to 6-8 month old beagles for 2 years at 0, 10, 50, 100 or 500 ppm, 3/sex/group. One low dose male died, all animals were necropsied and examined microscopically. No specific effect noted.

UNACCEPTABLE and does not appear to be upgradeable due to multiple deficiencies, including: no evidence of an MTD; no hematology, clinical chemistry, or urinalysis; no clinical observations; methods and evaluation criteria not detailed; no individual nor summary tabulated data included. The sponsor has requested EPA for copies of the full report which will be forwarded to CDFA for review (ref: document #142-111, letter dated 3/11/87). (D. Shimer/Y. Luthra, 12/2/87; re-examined without additional worksheet, C. Aldous, 8/10/88).

ONCOGENICITY, RAT
(See also combined rat, above)

142-111 055303 "Chronic Toxicity of 2,4-Dichlorophenoxyacetic Acid in Rats and Dogs." (Toxicol. Appl. Pharmacol. 20:122-129, 1971). Technical 2,4-D was given in the diet to Osborne-Mendel rats, 25/sex/group at 0, 5, 25, 125, 625 or 1250 ppm for 2 years. Survival was adequate and all animals were necropsied. Histopathology on 6/sex/group in high dose and control, and selected tissues of animals in other groups. Limited hematology done. No adverse effects indicated. UNACCEPTABLE, not upgradeable: too few animals on study, too few tissues systematically examined, no individual data, and other major variances from modern guidelines. The sponsor has requested EPA for copies of the full report which will be forwarded to CDFA for review (ref: document #142-111, letter dated 3/11/87). (D. Shimer/Y. Luthra, 12-2-87; re-examined without additional worksheet, C. Aldous, 8/10/88).

ONCOGENICITY, MOUSE

The data requirement has been met, with a possible adverse effect, based on possible hepatocellular effects indicated in the aborted portion of the Dow Chemical Study (Study ID: K-002372-063F), which tested males at and above the MTD. See in particular the review of the 1-year interim report of that study in Record No. 124245, below. That study indicated that males dosed with 150 to 300 mg/kg/day 2,4-D had increased hepatocellular tumors (incidences of 0/10, 0/10, 2/10, and 4/10 as of 1-year interim sacrifice). No hepatocellular tumor effect was observed in the more recent (2-year) study (Record No. 143336). Together, these studies indicate that 2,4-D elicits tumors at and above the MTD, but not below the MTD. The overall NOEL for non-neoplasia is 5 mg/kg/day, based on several studies below. Aldous, 2/24/00.

142-159 137060 Stott, W. T., K. A. Johnson, K. S. Gilbert, J. R. Ormand, J. E. Battjes, "2,4-Dichlorophenoxyacetic Acid: Dietary Oncogenicity Study in B6C3F1 Mice - Two year final report", The Dow Chemical Co., Midland MI, 3/10/95. Study ID: K-002372-063F. Mice were dosed with 2,4-D, 96.4% purity, in diets for 1 yr (10/sex/group) or 2 yr (50/sex/group) at dose levels of 0, 5, 150, or 300 mg/kg/day in diet. The present report concerns only results in females (see Record No. 124245 for limited results in males, which were terminated shortly after 1-yr interim sacrifice). NOEL = 5 mg/kg/day (kidney histopathology, particularly degeneration with regeneration of cortical tubules, and hypercellularity of the descending portion of the proximal tubules). The primary kidney findings constitute a "possible adverse effect", considering the

comparatively low NOEL. There is no oncogenic response in females. This segment of the mouse oncogenicity requirement (females only) is satisfactory. Aldous, 11/27/95. (See the following 1-liners for data on male mice).

142-176 156539 (supplemental histopathology for Record No. 143336, below). Spleens from term survivors of the low and intermediate dose levels were examined for histopathology, per U.S. EPA request. No additional tumors were found. DPR had not considered original report data to indicate tumor responses in the 1995 review. New data do not change the study status (no oncogenicity indicated in that study). Aldous, 12/30/99.

142-148 124245 Stott, W. T., Gilbert, K. S., Johnson, K. A., and Ormand, J. R.; "2,4-Dichlorophenoxyacetic Acid: Dietary Oncogenicity Study in B6C3F1 Mice - One Year Interim Report". The Toxicology Research Laboratory, HES, Dow Chemical Co., Midland, Michigan. Date of Director's signature on interim report: 5/21/93. Additional information on male mice is in the protocol attached to this report, under DPR Record No. 124244. Estimated achieved dosages were 5.2, 152.3, and 308.1 mg/kg/day for females. It is reasonable to presume that achieved dosages for males were similarly close to target. Apparent NOEL = 5 mg/kg/day [hypercellularity in descending portions of proximal tubules in females (kidney histopathology was not evaluated in males), slight (2 g) body weight decrement in males, elevated kidney weights in females and apparently in males (organ weight data were not tabulated for males), and slightly increased alopecia in females. hepatocellular adenomas in males]. Hepatocellular tumors are a "possible adverse effect". Body weights in 300 mg/kg/day males were reduced by over 5 g by 1-year into the study, exceeding the MTD range. Hepatocellular adenoma incidences in males at interim sacrifice (10/group) were 0, 0, 2, and 4 for controls through high dose, respectively. Registrant is requested to be more direct in reporting tumor treatment responses, even in discontinued portions of oncogenicity studies. **Not acceptable** (interim report, and data limited almost entirely to females). Useful information. Kishiyama and Aldous, 8/16/93.

**142-163 143336 Stott, W. T., K. A. Johnson, K. S. Gilbert, J. R. Ormand, J. E. Battjes, "2,4-Dichlorophenoxyacetic acid: Dietary oncogenicity study in male B6C3F1 mice - Two year final report", The Dow Chemical Co., Midland MI, 11/16/95. Study ID: K-002372-063MF. Fifty B6C3F1 male mice/group were dosed in diet with 2,4-D, purity 97.0 to 97.2%, at 0, 5, 62.5, or 125 mg/kg/day for 24 months. An additional 10/group were sacrificed for histopathology at 1 yr. NOEL = 5 mg/kg/day (slight increases in kidney weights; changes in kidney cortex, especially degeneration/regeneration of descending part of the proximal tubule). There was no effect on neoplasia, and no "adverse effects" were noted in this report. The present report, together with the final report on female mice (Record No. 137060) and the interim report containing limited information about effects on males at higher dose levels (Record No. 124245), suffice to fill the data gap for oncogenicity in mice. This report does not change the overall status of the mouse oncogenicity assessment [negative for females up to the MTD, evidence of positive effect for males at and above the MTD (i.e. 150 and 300 mg/kg/day, respectively), based on evidence of increased hepatocellular tumors at the latter dose levels]. Aldous, 1/16/96.

50721-049 112023 Protocol for Dow Chemical mouse dietary oncogenicity study. See interim report in Record No. 124245, above.

142-112 055305 "Oncogenicity study in mice with 2,4-dichlorophenoxyacetic acid (2,4-D)". Hazleton (Vienna, VA), 1/16/87. 2,4-D (97.5%) administered in diets of B6C3F1 BR mice at 0, 1, 15, and 45 mg/kg/day for 104 weeks. No oncogenic effect indicated. No adverse effect indicated, however risk assessment is appropriate due to the comparatively low NOEL in males only. NOEL = 1 mg/kg/day, based on changes in tubular epithelium (reduction of cytoplasmic vacuoles), dose related, at 15 and 45 mg/kg/day in males only. NOEL for females = 15 mg/kg/day (increased kidney weights at 53-week interim sacrifice without corresponding microscopic changes). NOT ACCEPTABLE, not upgradeable (Dose range not justified. Even though the NOEL in males was low, the minor kidney findings do not appear to be a limiting factor for the dosage range selection). ©. Aldous, 11/3/88).

142-120 071908 "Comments by the Technical Committee of the Industry Task Force on 2,4-D Research Data on the adequacy of the dose levels in the mouse oncogenicity and chronic rat studies with 2,4-D." Interpretative comments by the Technical Committee of the Industry Task Force on 2,4-D Research Data on the adequacy of dose levels in the mouse and rat long-term studies. These comments were submitted with a cover memo by John D. Conner, Jr. (Counsel to the Task Force) dated 6/3/88. Considered by C. Aldous with respect to mouse oncogenicity study 112:055305. See rebuttal document of 5/17/89.

142-120 071909 "Report on adequacy of high dose selection for 2,4-D oncogenicity studies". (May 26, 1988; CDFA record #071909). Considered by C. Aldous with respect to mouse oncogenicity study 112:055305. See rebuttal document of 5/17/89.

SUBCHRONIC (RANGE-FINDING FOR ONCOGENICITY), MOUSE

142-138 098299 Schulze, G. E., "Subchronic toxicity study in mice with 2,4-dichlorophenoxyacetic acid". Hazleton Laboratories America, Inc., Rockville, 8/16/91. 2,4-D, Lot # 909, purity 96.1% was given in diets of 10 B6C3F1 mice/sex/group at 0, 1, 15, 100, and 300 mg/kg/day for 13 wk. Major findings were nuclear hyperchromatism in livers of most high dose mice, and tubular degeneration in kidneys of most high dose males. Some clinical chemistry changes and relative kidney weight changes involved the higher two dose levels. Apparent NOEL was 15 mg/kg/day. No worksheet or formal acceptability evaluation was done at this time (study type is not required for SB-950). **No adverse effects** were indicated. Study supports proposed dose levels of 15, 150, and 300 mg/kg/day as treatment levels for an upcoming oncogenicity study. Aldous, 3/19/93.

142-139 093149 (Exact duplicate of 142-138:098299).

142-088 028525 Serota, D. G., "Subchronic toxicity study in mice: 2,4-dichlorophenoxyacetic acid (2,4-D)," Hazleton Laboratories America, Inc., Vienna, VA, 9/12/83, Project No. 2184-100. Twenty B6C3F1 mice/sex/group were dosed in diet at 0, 5, 15, 45, or 90 mg/kg/day for 3

months in a subchronic study. There were no effects on survival, body weight, or food consumption. The only apparent treatment effects were in the kidneys, namely (1) “increased homogeneity and altered tinctorial properties of the cytoplasm and decreased intracellular/intraluminal vacuolation in the cortex” (observed in males, at incidences of 1, 3, 9, 18, and 20 for controls through high dose groups, respectively), and (2) “increased homogeneity and altered tinctorial properties of the cytoplasm with or without cytoplasmic swelling in the cortex” (observed in females, at incidences of 1, 4, 6, 12, and 14 for controls through high dose groups, respectively). Thus it appears that a NOEL for these observations would be under 5 mg/kg/day in either sex. Aldous, 8/8/06 (no worksheet).

REPRODUCTION, RAT

The most recent of the two following reproduction studies (Vols. 100-104) is by far more consistent with modern guidelines than the older study. Both studies indicated "possible adverse effects" (marked gestational and neonatal losses at comparable dose levels: 80 mg/kg/day is approximately equivalent to 1500 ppm in rat studies). Both studies suggested comparable NOEL’s (20 mg/kg/day vs 500 ppm). The NOEL from the acceptable study (100:047265) is more reliable for possible risk assessment. Aldous, 11/04/88.

****142-100** to **-104 047265-047269** "Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxyacetic Acid.” (7/26/85, WIL Research Laboratories, Inc. Project No. WIL-01137). 2,4-D, 97.5% administered in diet to 30/sex/group at nominal levels of 0, 5, 20 or 80 mg/kg/day - 2 litters, 2 generations. High dose was discontinued after weaning of F1b litters due to severe prenatal and neonatal losses. Because of these losses, the study was considered by CDFA to indicate a **possible adverse reproductive effect** at 80 mg/kg/day nominal dosage to the F0 parents (J. Gee, 9/24/86). Actual administered dosages were appreciably higher than nominal during much of gestational and lactation periods; elevated as much as 66% over target levels at times in the high dose group. Parental and reproductive effects Noel’s were 20 mg/kg/day, based on maternal weight losses or weight gain decrements during gestation and lactation, and also upon reduced gestational and neonatal survival (F1b litter 80 mg/kg/day group gestation survival was 31.7%, with total litter losses in 15 high dose group F0 dams). The 11/3/88 review did not change study status (acceptable, possible adverse effects), but confirmed that there was some maternal body weight effect attributable to treatment at the high dose. The adverse effect indication was <u>not</u> removed, since markedly reduced gestational and neonatal survival was observed, which was <u>not</u> accompanied by a commensurate degree of maternal toxicity. (J. Gee, 9/24/86, C. Aldous, 11/3/88.)

142-111 [no record number] Industry Task Force rebuttal, submitted 3/11/87, referring to 9/24/86 CDFA review; no new data.

142-089 028385 Interim report to 100:047265, above.

142-111 055303 "Chronic Toxicity of 2,4-Dichlorophenoxyacetic Acid in Rats

and Dogs." (Toxicol. Appl. Pharmacol. 20:122-129, 1971). 2,4-D technical was administered to rats in a three generation, 2 litters/generation reproduction study at 0, 100, 500 or 1500 ppm in the diet. Ten males and 20 females/group. Report does not discuss parental toxicity (except to note that fertility was not affected). Apparent reproductive effects NOEL = 500 ppm (reduced pup survival and weanling weight was observed at the high dose). UNACCEPTABLE**,** not upgradeable: No individual data, no necropsy or histopathology, no mating records, no evaluation of possible parental toxicity, relevant parameters not reported (such as birth weights, gestation time, and various reproductive indices), and other major variances from guidelines. Only one data table provided. The sponsor has requested EPA for copies of the full report which will be forwarded to CDFA for review (ref: document #142-111, letter dated 3/11/87). (D. Shimer/Y. Luthra, 12/3/87; re-examined without additional worksheet, C. Aldous, 8/10/88).

TERATOLOGY, RAT

142-099 047264 Nemec, M. D., E. J. Tasker, K. M. Werchowski, and M. D. Mercieca, "A Teratology Study in Fischer 344 Rats with 2,4-dichlorophenoxyacetic acid", (WIL Research Laboratories, Inc., 3/2/83). 2,4-D acid (97.5%) administered by gavage in corn oil to 35 female Fischer 344 rats per group at 0, 8, 25, and 75 mg/kg/day. Study was initially classified as unacceptable but upgradeable on receipt of dosing solution analysis, and a "possible adverse effect" was attributed to slight indications of delayed ossification (review of J. Gee, 9/25/86). The latter review placed maternal and developmental effects Noel's at 75 and 25 mg/kg/day, respectively. The report was re-examined in response to the 3/11/87 rebuttal submission (Document 142-111, Enclosure 2, no Record #). The August, 1988 review concluded that the study is ACCEPTABLE, and that there is **no adverse effect, and that the maternal and developmental effects Noel's were both 25 mg/kg/day. ©. Aldous, 8/15/88.)

EPA 1-liner: Maternal, teratogenic NOEL > 75 mg/kg (HTD) Fetotoxic NOEL= 25 mg/kg. Fetotoxic LEL = 75 mg/kg (for delayed ossification)

142-089 028384 Incomplete version of final report (099:047264).

142-089 028386 "A range-finding teratology study in Fischer 344 rats with 2,4-dichlorophenoxyacetic acid". A retrospective dose range-finding study for 099:047264. WIL Research Laboratories, Inc., 5/17/83. Levels tested by gavage in Fischer 344 rats: 0, 75, 100, 150, 200 and 250 mg/kg in corn oil, days 6-15 of gestation. There were 1/10 and 3/10 deaths at the 200 and 250 mg/kg/day levels with deaths attributed to cerebral hemorrhage. At 200 and 250 mg/kg/day, there were total litter losses in all dams. Two of 8 pregnant dams at 150 mg/kg/day had total litter losses. These losses are considered to be treatment effects. Maternal body weight gains were clearly diminished at 150 mg/kg/day and above, and very marginally diminished at 75 and 100 mg/kg/day. There is thus an equivocal LEL of 75 mg/kg/day to support dose selection of the primary study (099:047264); however CDFA reviewers, J. Gee, Y. Luthra, and C. Aldous all have indicated that a somewhat higher maximum dosage would have been preferable in the primary study. [Reviews by Gee, 9/10/85 and 9/29/86, (study examined

by Y. Luthra in Dec., 1987 without separate written review), C. Aldous, 8/3/88 (included in re-examination of 099:047264)].

EPA 1-liner: Maternal LEL = 150 mg/kg (reduction in food consumption & body weight loss; Maternal NOEL = 100 mg/kg.

142-132 095866 Lochry, E. A., "Developmental toxicity (Embryo-fetal toxicity and teratogenic potential) study of 2,4-D dimethylamine salt (2,4-D-DMA) administered orally via gavage to Crl:CD®BR VAF/Plus® presumed pregnant rats". Argus Research Laboratories, Inc. (Protocol No. 320-001), 11/15/90. Pregnant rats, about 105 days old, were allocated to groups of 25 at dose levels of 0, 12.5, 50, and 100 mg/kg/day [free acid equivalent] of 2,4-D-DMA. Treatment by gavage with aqueous solutions was done daily from days 6 to 15 of gestation. **No adverse effects were indicated. Maternal NOEL = 12.5 mg/kg/day (minor body weight decrements associated with a minor decrease in food consumption during treatment at 50 mg/kg/day). At 100 mg/kg/day, there were greater decrements in body weight gain and food consumption, and several dams displayed ataxia and decreased motor activity. Developmental NOEL = 50 mg/kg/day (slight, but statistically significant reduction in fetal body weights; increased skeletal alterations, including wavy and/or incompletely ossified ribs, incompletely and/or unossified sternebrae). A pilot study (reported in this record) had shown increased early resorptions and "small or absent eye bulges" at 200 mg/kg/day, along with more marked maternal toxicity. The primary study did not elicit any malformations up to 100 mg/kg/day, nor did that dose increase resorptions. Study is **acceptable** (previously considered unacceptable, based on insufficient characterization of test article, and a need for evidence of stability of dosing solutions under test conditions). See Record No. 112021 for data which allowed an upgrade of the primary study. Aldous, 7/30/91, 3/19/93.

50721-047 112021 [Requested information about stability of dosing material]. Additional data were provided. Argus Research Corp. performed retrospective sample preparation, and analysis was provided by Lancaster Laboratories, Inc. Date of these additional data: 12/23/91. The original review (under Record No. 095866) had requested (1) further characterization of test article, and (2) evidence of stability of dosing solutions. The present submission addresses both issues, allowing a change of report status to **acceptable**. Test article, Lot # 04FD31349, is tech. 2,4-D dimethylamine salt (66.18% A.I., 55.5% 2,4-D acid equivalent). It is manufactured at only one location, and the retrospective studies performed at Argus Laboratories used comparable material from the same source. Argus Laboratories prepared samples in the same manner as was done in the original study, and submitted freshly prepared samples, as well as other samples stored at RT for 7 days, at concentrations corresponding to low and high dose levels of the original study. There was no decomposition during the 7-day period. Aldous, 3/19/93.

142-090 028387 "Teratology Study in Fischer 344 Rats with 2,4-Dichlorophenol, Final Report." (3/31/83, WIL). 2,4-Dichlorophenol, metabolite of 2,4-D. Purity not stated. 34 females per group were given 0, 200, 375 or 750 mg/kg/day by oral gavage, days 6 - 15. NOTE: Tables 9 - 18 (individual data) are not included with the report. UNACCEPTABLE in support of 2,4-D

data requirements, but useful data. Four out of 34 dams died in the 750 mg/kg/day group. Only this high dose group had quantifiable evidence of maternal toxicity, largely limited to body weight gain decrements, which were statistically and probably biologically significant. Weight gain decrements in other groups were occasionally statistically significant, but very small in magnitude and of no apparent physiological significance. Fetal findings were not treatment-related, except possibly for slightly decreased ossification of sternebrae #1 - #4 and of vertebral arches in the 750 mg/kg/day group only. Original review by J. Remsen (Gee) indicated "possible adverse [reproductive] health effects", citing decreased maternal body weights (albeit usually very small decrements) at all doses [J. R. (Gee), 9/10/85]. Status changed to supplemental study, with no indication of adverse effects [Y. Luthra (no written review), 4/1/88; and C. Aldous, 11/3/88]. [Note: no additional data are presently required of this study].

TERATOLOGY, RABBIT

142-131 095865 Hoberman, A.M., "Developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of 2,4-dichlorophenoxyacetic acid (2,4-D acid) administered orally via stomach tube to New Zealand White rabbits". Argus Research Laboratories, Inc., Project No. 320-003, 12/12/90. Twenty artificially inseminated dams/dose were administered 0, 10, 30, or 90 mg/kg/day 2,4-D technical (96.1%) by gavage in aq. 0.5% methylcellulose on days 6-18 of gestation. **No adverse effects. Maternal-fetal NOEL = 30 mg/kg/day (2/18 high dose dams had abortions). Developmental NOEL = 30 mg/kg/day (in addition to abortions, noted above: the percentage of males among live fetuses was substantially higher in the 90 mg/kg/day group than in other groups). A pilot study (same record) showed high mortality to does at 200 mg/kg/day, and it also appeared that 100 mg/kg/day caused one abortion and necessitated one moribund sacrifice out of a group of 4 does: indicating a steep dose-response curve for maternal toxicity. **Acceptable**, Aldous, 7/30/91.

TERATOLOGY, HAMSTER

142-111 055304 "Teratogenic studies with 2,4,5-T and 2,4-D in the hamster", FDA, Washington, D.C., in Bull. Environ. Contam. Toxicol. 6 (6):559-567 (1971). 2,4-D, either Dow Chemical Co. "Tech.", Dow Chemical Co. "Current Production", or Hercules Powder Co. "Current Production"; 7 to 12 golden Syrian hamster dams/dose/test article dosed by gavage on days 6-10 at dosage levels of 100, 60, 40, and (in case of Dow "Tech" only) 20 mg/kg/day. There were 86 control dams. There was no reported maternal toxicity. A possible increase in fused ribs over control levels was suggested at 60 to 100 mg/kg/day, however group sizes were too small and data too limited for meaningful evaluation. No adverse effects indicated, considering the limited evidence of a treatment effect, and the appreciable dose level of the apparent NOEL. Study is UNACCEPTABLE, and not upgradeable. No further information is required of this report. ©. Aldous, 8/8/88).

GENE MUTATION

** 142-011 086185, "Mutagenicity Test on 2,4 Dichlorophenoxyacetic Acid in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test)", (T. E. Lawlor and D. C. Valentine, Hazleton Laboratories America, Study No. 10979-0-401, 2/26/90). 2,4 Dichlorophenoxyacetic Acid, purity 96.1%, was tested at concentrations of 0 (DMSO), 100, 333, 667, 1000, 3330, or 6670 µg/plate with metabolic activation (Aroclor 1254-induced rat liver) and at 0, 66.7, 100, 333, 677, 1000, or 3330 µg/plate without metabolic activation. In a confirmatory test, 2,4-D was tested at 0, 333, 667, 1000, 3330, 6670, or 10000 µg/plate with metabolic activation and at 0, 100, 333, 667, 1000, 3330, or 6670 µg/plate without S9 Mix. Assayed with Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538. Incubation time was for 48 hours. In both the initial and confirmatory assays, 2,4-dichlorophenoxyacetic Acid treatments did not significantly increase the number of revertants. **Negative and acceptable.** (Kishiyama and Gee, 7/26/91)

** 142-097 047257 Salmonella. "Mutagenicity Testing of Agent Orange Components and Related Chemicals (Salmonella typhimurium) (5/18/84, Journal article in: Toxicol. Appl. Pharmacol. 75:137-146). 2,4-D, 99%; Salmonella TA 1535, TA 1537, TA98, TA100 ± rat and hamster liver activation; 2,4-D and related compounds at 0, 33, 100, 333, 1000, 3333 or 10,000 µg/plate after 20 min preincubation; no increase in reversion rate; triplicate plates - 2 labs ACCEPTABLE.
(Gee, 9-16-86).

** 50721-033 086186, "Mutagenicity Test on 2,4-D Dimethylamine Salt in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test)", (T. E. Lawlor and D.C. Valentine, Hazleton Laboratories America, Study No. 10981-0-401, 2/26/90). 2,4-D dimethylamine salt, purity 66.18%, at concentrations of 0 (deionized water), 333, 667, 1000, 3330, 6670, or 10000 µg/plate without and with metabolic activation (Aroclor 1254-induced rat liver) was assayed with Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 by plate incorporation. Incubation time was 48 hours. 2,4-D Dimethylamine salt treatments in both the initial and repeat assays did not significantly increase the number of revertants. Evaluated as negative and unacceptable but upgradeable with further characterization of the test material. (Kishiyama and Gee, 7/29/91) A description of the test material is contained in 50721-048, record # 112026. The study is upgraded to acceptable status. Gee, 8/6/93.

50721-048 112026 Supplement to 086186. Contains a description of the test article and upgrades the study to acceptable status. Gee, 8/6/93.

** 50730-003 086187, "Mutagenicity Test on 2,4-D, 2-Ethylhexyl Ester in the Salmonella/Mammalian-Microsome Reverse Mutation Assay (Ames Test)", (T. E. Lawlor and D.C. Valentine, Hazleton Laboratories America, Study No. 10980-0-401, February 26, 1990). 2,4-D, 2-Ethylhexyl Ester [grouped with 2,4-D free acid as of 7/23/91], purity of 98.0%, at concentrations of 0 (DMSO), 333, 667, 1000, 3330, 6670, or 10000 µg/plate without and with

metabolic activation (Aroclor 1254-induced rat liver) was assayed with Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538. Incubation period was for 48 hours. 2,4-D,-2-Ethylhexyl Ester did not increase the number of revertants in either the initial or repeat assay. **Negative and acceptable.** (Kishiyama and Gee, 7/23/91)

142-097 047258 "Evaluation of Herbicides for Possible Mutagenic Properties (Salmonella typhimurium) Salmonella (1972, Publ.: J. Agr. Food Chem. 20(3): 649 (1972). Salmonella and T_4 phage; 50 µg only; no data, negative effect; Incomplete and UNACCEPTABLE. (Gee, 9-16-86).

142-097 047261 J.P. Seiler, "The genetic toxicology of phenoxy acids other than 2,4,5-T". Mutation Research 55:197-226 (1978). A review article without "reviewable" data. No Med Tox review. (Aldous, 7/31/91).

142-097 047262 2-pg discussion of mutagenicity, with no reviewable data. No Med Tox review. (Aldous, 7/31/91).

CHROMOSOME EFFECTS

** 142-011 086188, "Mutagenicity Test on 2,4-Dichlorophenoxyacetic Acid In Vivo Mouse Micronucleus Assay", (J. L. Ivett, Hazleton Laboratories America, Study No. 10979-0-455, 2/27/90). 2,4-Dichlorophenoxyacetic acid, purity 96.1%, administered as a single dose (gavage) at 0 (corn oil), 40, 133, or 400 mg/kg to 5 ICR mice/sex/group. Bone marrow polychromatic erythrocytes were harvested at 24, 48, and 72 hours after administration. Negative controls were harvested at 24 hours only. The test substance did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes. **Negative and acceptable.** (Kishiyama and Gee, 7/26/91)

142-127 086231, "Single Acute Exposure Dose Selection Study on 2,4 - Dichlorophenoxyacetic Acid", (J. L. Ivett, Hazleton Laboratories America, HLA Study No. 10979-0-459-PO, 2/27/90). 2,4 - Dichlorophenoxyacetic Acid, purity 96.1%, administered by gavage (single dose) at concentrations of 300, 675, 1050, 1425, or 1800 mg/kg to 3 ICR mice/sex/group. $_{LD50}$ = 563 mg/kg for both males and females. Mortality was within 3 days. This study provided satisfactory information for selection of doses for a subsequent in vivo bone marrow micronucleus assay (above). (Kishiyama and Gee, 7/30/91)

** 50721-033 086189, "Mutagenicity Test on 2,4-D Dimethylamine Salt In Vivo Mouse Micronucleus Assay", (J. L. Ivett, Hazleton Laboratories America, Study No. 10981-0-455, 2/27/90). 2,4-D Dimethylamine Salt, purity 66.18%, administered a single dose (gavage) at concentrations of 0 (deionized water), 60, 200, or 600 mg/kg (not adjusted for purity) to 5 Sprague-Dawley ICR mice/sex/group. Bone marrow polychromatic erythrocytes were harvested at 24, 48, and 72 hours after administration. The test substance did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes. Evaluated as negative, unacceptable but possibly upgradeable with further information on the test material.

(Kishiyama and Gee, 7/25/91) The requested information on the test material was submitted in 50271 048 112026 and the study is upgraded to **acceptable** status. Gee, 8/6/93.

50271-048 112026 Supplemental data for 086189 containing a description of the test material used. The study has been upgraded to acceptable status. Gee, 8/6/93.

50721-034 086235, "Single Acute Exposure Dose Selection Study on 2,4-D Dimethylamine Salt", (J. L. Ivett, Hazleton Laboratories America, Study No. 10981-0-459-PO, 2/27/90). 2,4-D dimethylamine salt, purity 66.18%, administered by gavage (single dose, intubation) at concentrations of 400, 800, 1200, 1600, or 2000 mg/kg to 3 ICR mice/sex/group. $_{LD50}$ = 976 mg/kg (95% confidence limits 739 and 1209 mg/kg) for males and females, combined. The report does not indicate if purity was considered. Mortality was within 3 days. This study provides information for the selection of doses for a subsequent in vivo bone marrow micronucleus assay (above). (Kishiyama and Gee, 7/29/91)

** 50730-003 086190, "Mutagenicity Test on 2,4-D, 2-Ethylhexyl Ester In Vivo Mouse Micronucleus Assay", (J. L. Ivett, Hazleton Laboratories America, Study No. 10980-0-455, 2/27/90). 2,4-D, 2-Ethylhexyl Ester, purity 98.0%, LOT # 04KF54479, was administered as a single dose by gavage at 0 (corn oil), 50, 167, or 500 mg/kg to 5 ICR mice/sex/group. Bone marrow was harvested at 24, 48, and 72 hours after dosing. Polychromatic erythrocytes were scored for micronuclei and the PCE/NCE ratio determined. One thousand PCE's were scored per animal. The test substance did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes. **Negative and acceptable.** (Kishiyama and Gee, 7/24/91)

50730-004 086236, "Single Acute Exposure Dose Selection Study on 2,4 - D - 2 - Ethylhexyl Ester", (J. L. Ivett, Hazleton Laboratories America, Study No. 10980-0-459-PO, 2/27/90). 2,4 - D - 2 - Ethylhexyl Ester, purity 98.0%, administered by gavage (single dose, intubation) at concentrations of 400, 800, 1200, 1600, or 2000 mg/kg to 3 ICR mice/sex/group. $_{LD50}$ = 673 mg/kg for both males and females. Mortality was noted within 3 days. This study provides information for the selection of doses for a subsequent in vivo bone marrow micronucleus assay (above). Supplemental data. (Kishiyama and Gee, 7/25/91)

142-097 047259 "Distribution and Cytogenetic Test of 2,4-D and 2,4,5-T Phenoxyacetic Acids in Mouse Blood Tissues." (1976, Publ.: Chem.-Biol. Interactions 14:291-92, 2,4-D Task Force, Wallenberg Lab, Stockholm) Male CBA mice, 3 at 100 mg/kg, sacrificed at 24 hours or 7 days; i.p. injection; some decrease in % PCE's at 24 hours (not at 7 days); no increase in micronuclei; UNACCEPTABLE - males only, times of sacrifice, other deficiencies. (Gee, 9-16-86).

DNA DAMAGE

** 142-011 086191, "Mutagenicity Test on 2,4-Dichlorophenoxyacetic Acid (2,4-D) in the In Vitro Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay", (M. A. Cifone, Hazleton Laboratories America, Inc., Study No. 10979-0-447, 2/28/90). 2,4-Dichlorophenoxyacetic Acid,

purity 96.1%, at concentrations of 0 (DMSO), 2.42, 4.85, 9.69, 24.2, 48.5, or 96.9 µg/ml, was assayed with primary rat hepatocytes. Treatment period was 18 hours. 2,4-Dichlorophenoxyacetic acid did not induce unscheduled DNA synthesis. **Negative and acceptable.** (Kishiyama and Gee, 7/26/91)

** 50721-033 086192, "Mutagenicity Test on 2,4-D Dimethylamine Salt in the In Vitro Rat Primary Hepatocyte Unscheduled DNA Synthesis", (M.A. Cifone, Hazleton Laboratories America, Inc., Study No. 10981-0-447, 2/28/90). 2,4-D Dimethylamine Salt, purity 66.18%, at nominal concentrations of 0 (deionized water), 2.5, 5.0, 10.0, 25.0, 50.0, or 100.0 µg/ml were assayed with primary rat hepatocytes. Concentrations were not adjusted for purity. Treatment period was 18.3 hours. 2,4-D Dimethylamine did not induce unscheduled DNA synthesis under the test conditions. Evaluated as unacceptable but possibly upgradeable. No individual data (see worksheet). (Kishiyama and Gee, 7/26/91) With the submission of 048 112027, the study is upgraded to acceptable status. Gee, 8/6/93.

50721-048 112027 Supplement to 033 086192. Contains an explanation about the test article, historical controls for $\geq$ 5 net nuclear grains and the individual slide data, upgrading the study to acceptable status. No worksheet. Gee, 8/6/93.

** 50730-003 086193, "Mutagenicity Test on 2,4-D, 2-Ethylhexyl Ester in the In Vitro Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay", (M.A. Cifone, Hazleton Laboratories America, Inc., Study No. 10980-0-447, 2/28/90). 2,4-D, 2-Ethylhexyl ester, 98.0% purity, at concentrations of 0 (DMSO), 0.501, 1.00, 2.50, 5.00, 10.0, or 25.0 µg/ml, was assayed with primary rat hepatocytes. The treatment period was 19 hours. 2,4-D, 2-Ethylhexyl ester, did not induce unscheduled DNA synthesis. **Negative and acceptable.** (Kishiyama and Gee, 7/24/91)

142-097 047260 "Phenoxyacids as Inhibitors of Testicular DNA Synthesis in Male Mice." (2,4-D Task Force, 1979 publ. in Bull. Environm. Contam. Toxicol. 21: 89-92, J. P. Seiler.) 2,4-D, purity not stated. DNA synthesis in male mice (number not given); 200 mg/kg orally, 3-5 hours; radioactive thymidine incorporated into testicular DNA was measured. **Possible adverse effect indicated:** Inhibition of testicular DNA synthesis of 29%. Incomplete, UNACCEPTABLE. (Gee, 9-16-87).

Note: The initial evaluation considered there to be a possible adverse effect. This was based, in part, on a 2,4-DB study erroneously submitted with 2,4-D data. The 1-liner for that study has now been deleted from the 2,4-D Summary of Toxicology Data. There remains one inadequate study (DNA synthesis inhibition, Record No. 047260) with a possible adverse effect. There are, however, several negative studies in the "DNA Effects" classification which are considered to be acceptable. These negative, acceptable studies do not allow the positive study to be ignored, since the positive study was fundamentally different in design and represented an in vivo mammalian finding. There remains "possible adverse effect" for "DNA Effects". J. Gee, 8/12/93.

NEUROTOXICITY

Hen neurotoxicity studies are not required at this time. Summaries of rat studies follow.

NOTE: A negative rat study was submitted, which did not indicate effects in grip strength or in microscopic evidence of central or peripheral nervous system damage following repeated dermal exposure to 2,4-D dimethylamine salt. No worksheet performed nor required ©. Aldous, 8/11/88, Vols. 087 and 098, Record # 028382, and duplicate record #047263). U.S. EPA requested additional rat neurotoxicity studies in the Guidance Document of Sept. 1988. Studies below appear to have been in response to that request.

142-156 132078 Mattsson, J. L., R. J. McGuirk, and B.L. Yano, "2,4-Dichlorophenoxyacetic acid (2,4-D): Acute neurotoxicity study in Fischer 344 rats", The Dow Chemical Co., Midland MI, Jan. 5, 1994. Laboratory Project Study ID# K-002372-066. Ten rats/sex/group were dosed once by gavage with 2,4-D (96.6%) in corn oil at 0, 15, 75, or 250 mg/kg. Motor activity and FOB were assessed on days -1, 1 (6 hr after treatment), 8, and 15. During the first few days after treatment, rats received detailed clinical examinations. Five/sex/group were then necropsied on day 15 following in situ perfusion, and examined microscopically for nervous system damage. High dose rats commonly showed incoordination (awkward placement of paws) and abnormal gait (slight knuckling of forepaws) during the FOB on day 1. These signs steadily decreased over the next 3 days, and were not seen thereafter. Two 75 mg/kg rats also had an awkward gait on day 1 (only one of these, a female, showed sufficient change to be considered clearly treatment-related). Motor activity of high dose rats was sharply decreased on day 1 only. Other in vivo measures were negative. NOEL = 15 mg/kg (slight gait abnormalities on day 1 only). There were no histopathologic changes. Study is **acceptable, with no adverse effects. Aldous, 12/11/95.

****142-157 132079** Mattsson, J. L., T. K. Jeffries, and B.L. Yano, "2,4-Dichlorophenoxyacetic acid (2,4-D): Chronic neurotoxicity study in Fischer 344 rats", The Dow Chemical Co., Midland MI, 6/28/94, Laboratory Project Study ID# K-002372-064N. Ten rats/sex/group were assigned to the neurotoxicity portion of a chronic study. Rats received 0, 5, 75, or 150 mg/kg/day 2,4-D (96.4%) for 1 yr. Motor activity and FOB were assessed pre-exposure, and at months 3, 6, 9, and 12. Five/sex/group were necropsied at 12 months, following in situ perfusion, and controls and high dose rats were examined microscopically for nervous system damage. Histopathology examinations included eyes (retina and optic nerve), for which intermediate groups were examined because high dose effects were found. NOEL = 5 mg/kg/day (modest, dose-related decrements in body weights were observed at 75 to 150 mg/kg/day). A slight increase in urine quantity in high dose females was possibly treatment-related. The major finding germane to neurotoxicity evaluation was bilateral retinal degeneration in 150 mg/kg/day females (a "possible adverse effect"). The NOEL for neurotoxicity = 75 mg/kg/day. Study is **acceptable**. Aldous, 12/04/95.

METABOLISM

142-133 095867 Timchalk, C., Dryzga, M.D., and Brzak, K. A., "2,4-Dichlorophenoxyacetic acid, tissue distribution and metabolism of 14C-labeled 2,4-dichlorophenoxyacetic acid in Fischer 344 rats". Dow Chemical Co., Midland, MI, Dec. 5, 1990. Rats were dosed once with 1 or 100 mg/kg labeled 2,4-D; or once daily with 1 mg/kg/day unlabeled 2,4-D for two weeks followed by a single dose of 1 mg/kg of labeled 2,4-D; or with a single iv dose of 1 mg/kg of labeled 2,4-D. Regardless of route, dose level, or duration of treatment; most of the dose was excreted in urine. Almost all of the 2,4-D was excreted unchanged. Peak plasma levels were obtained after about 4 hr, and very little remained in tissues at 48 hr. Data suggest rapid absorption and rapid excretion. No Medical Toxicology Branch review worksheet was made as of 6/28/91 (Aldous).

142-0225 204130 Hardwick, T., "The pharmacokinetics of (^{14}C)-2,4-D in the rat and dog," Covance Laboratories Ltd, North Hampshire, England, Oct. 11, 2002. Covance Report # 1149/40-D1145. Beagle dogs and F-344 rats were dosed once, generally in groups of 4/sex/dose/parameter, at 5 and at 50 mg/kg/day of (^{14}C)-2,4-D (ring label). Dogs were dosed by via carboxymethylcellulose (CMC) in gelatine capsules. Rats were dosed by gavage in 1% CMC. Investigators evaluated label in blood, plasma, urine, feces, cage wash, and (in the case of rodents) carcass. Dogs were not evaluated for residual radiolabel at termination. In addition to determination of kinetic parameters, the report shows several HPLC radiochromatograms of urine and plasma samples. It appears that parent compound was the dominant labeled component in rat urine and the only recognized peak in dog plasma. There were typically several peaks in dog urine, with the presumed parent compound present, but as not the dominant peak. The characterization of metabolites is presented separately (DPR Document No. 142-0226, Record No. 204131, Covance Report # 1149/042-D1145). Supplementary data. Aldous, 8/14/06.

142-0226 204131 Hardwick, T., "(^{14}C)-2,4-D: metabolite identification in the rat and dog," Covance Laboratories Ltd, North Yorkshire, England, Feb., 2003. Covance Report #1149/042-D1145. This study primarily evaluated urine samples from selected 2,4-D-treated animals in the associated Record No. 204130, specifically two F-344 rats (1/sex) dosed at 50 mg/kg/day 24 hr before collection, and two beagle dogs/sex/group dosed at 5 and 50 mg/kg/day, and sampled at 12 hr and 24 hr after dosing. In addition, 8-hr plasma samples were analyzed from 4 male dogs in that study dosed with 50 mg/kg/day 2,4-D. Analysis was by HPLC-MS, with HPLC effluent split between MS and UV and/or radiodetector. Where relevant, investigators used MS/MS and MS/MS/MS analyses to further characterize metabolites. Rat urine and dog plasma radiolabel appeared to be almost 100% parent 2,4-D. Dog urinary metabolite patterns varied greatly between individuals. Dog urine usually contained appreciable parent 2,4-D (3 to 16% of administered dose), but normally other metabolites were more abundant. Most of the more abundant metabolites were conjugates that preserved both chlorines on the phenyl ring as well as the phenoxyacetate. Commonly the major dog urinary metabolite was the glycine conjugate of 2,4-D (1 to 34% of administered dose). Occasionally the glucuronide conjugate of 2,4-D was more abundant (0.5 to 7% of administered dose), seemingly increasing in relative abundance over time. Somewhat less common was the taurine conjugate of 2,4-D (less than 1% of

administered dose). Several very minor metabolites were identified. Useful supplementary data. Aldous, 8/15/06.

U.S. EPA Reviews Cross-referenced to DPR Record Numbers (all study types)

Contents of the following brief reports in Document No. 142-177 were summarized without formal analysis by C. Aldous on 1/15/99.

142-177 156540 U.S. EPA executive summary for reviews of rat and mouse studies. **(1)** Rat study is DPR Document No. 142-175, Record No. 156537. Jeffries, T. K, B. L. Yano, J. R. Ormand, and J. E. Battjes, "2,4-Dichlorophenoxyacetic acid: Chronic toxicity/oncogenicity study in Fischer 344 rats", The Dow Chemical Company, Midland, MI, 3/28/95. Laboratory Project Study No. K-002372-064. EPA MRID No. 43612001. **(2)** Male mouse oncogenicity study is DPR Document No. 142-163, Record No.143336: Stott, W. T., K. A. Johnson, K. S. Gilbert, J. R. Ormand, J. E. Battjes, "2,4-Dichlorophenoxyacetic acid: Dietary oncogenicity study in male B6C3F1 mice - Two year final report", The Dow Chemical Co., Midland MI, 11/16/95. Study ID: K-002372-063MF. EPA MRID No. 43879801. **(3)** Female mouse oncogenicity study is DPR Document No. 142-159. Record No. 137060: Stott, W. T., K. A. Johnson, K. S. Gilbert, J. R. Ormand, J. E. Battjes, "2,4-Dichlorophenoxyacetic Acid: Dietary Oncogenicity Study in B6C3F1 Mice - Two year final report", The Dow Chemical Co., Midland MI, 3/10/95. Study ID: K-002372-063F. EPA MRID No. 43597201.

142-177 156541 U.S. EPA executive summary for reviews of rat developmental toxicity study: DPR Document/Record Nos. 142-099 047264: Nemec, M. D., E. J. Tasker, K. M. Werchowski, and M. D. Mercieca, "A Teratology Study in Fischer 344 Rats with 2,4-dichlorophenoxyacetic acid", (WIL Research Laboratories, Inc., 3/2/83). EPA MRID No. 000251031.

142-177 156542 U.S. EPA tabular summary of major FIFRA studies and results.

142-177 156543 U.S. EPA Data Evaluation Record (DER) of the rat combined study of 3/28/95: Record No.156537 = EPA MRID No. 43612001.

142-177 156544 U.S. EPA DER's of recent male and female mouse oncogenicity studies: Male mouse oncogenicity study is Record No. 143336 = EPA MRID No. 43879801. Female mouse oncogenicity study is Record No. 137060 = EPA MRID No. 43597201.

142-177 156545 U.S. EPA DER of rat developmental toxicity study: DPR Document/Record Nos. 142-099 047264 = EPA MRID No. 000251031.

142-177 156546 U.S. EPA "Carcinogenicity Peer Review" for 2,4-D, dated 1/29/97. (Conclusion: continue to classify as Group D - not classifiable as to human carcinogenicity). This document is of possible value for risk assessment, since it reviews actions of U.S. EPA.

142-177 156547 U.S. EPA RfD/Peer Review Report of 2,4-D (May 9, 1996). This record evaluated the usefulness of the major FIFRA studies for setting RfD values. This report settled on using the NOEL of 1 mg/kg/day from the dog study (DPR Record No. 127497 = EPA MRID No. 43049001: Hazleton 1993 study). Using an uncertainty factor of 100, the RfD was calculated to be 0.01 mg/kg/day.

142-177 156548 U.S. EPA "Toxicology Endpoint Selection Document". Document discusses various durations of potential exposure to 2,4-D, and proposes corresponding NOEL's. The dog study (see Record No. 156547) continues to be the basis of the RfD.

VARIOUS SUBMISSIONS RECEIVED: NOT PRIMARY STUDIES

142-0200 165382 "2,4-Dichlorophenoxyacetic acid (2,4-D): An update of the 2,4-D Cohort Mortality Study (letter in reference to FIFRA §6(a)(2) data from R. A. McCormick representing Dow AgroSciences LLC), 10/30/98. A study of illnesses and injuries of all Dow Midland employees with potential 2,4-D experiences compared health findings against expected incidences. Two illnesses were slightly elevated over norms: non-Hodgkin's lymphoma (3 deaths vs. 1 expected), and ALS (Lou Gehrig's disease: 3 deaths vs. less than 1 expected). Dow determined that these were incidental. Dr. Michael O'Malley of DPR Worker Health and Safety Branch also evaluated the extent and durations of associated exposures, and he also concluded that these events were not attributable to exposure. Aldous, 7/12/06 (no worksheet).

142-0230 203961 Timchalk, C., "Lack of Relevance of Toxicology Findings in Dogs for Assessment of Potential Human Health Risks of 2,4-D: A White Paper by the Industry Task Force II on 2,4-D Research Data," Battelle Pacific Northwest Laboratories, Richland, WA, 01/31/2003. Various parameters were examined across species for 2,4-D and for its congener, MCPA. Plasma half-life (hrs) for several species were 0.75 (mouse), 1.1 to 2.1 (rat), 31 or 91-106 (dog: 2 different studies), 6.6 (pig), 5 (calf), and 11.6 (human). Allometric evaluations of the half-life vs. b.w. or clearance vs. b.w. of either of these weak organic acids showed that humans and all species above but dog showed linear curves in log-log comparisons. Dogs had conspicuously longer plasma half-life and slower plasma clearance. Aldous, 7/12/06 (no worksheet).

142-0236 222050 Record appeared on 2,4-D search, but has no toxicity information.

50515-0061 125835 "17-day Cataractogenic Study with USB 3153 Technical in White Leghorn Chicks," Industrial Bio-test Laboratories Inc. (IBT study of unknown acceptability). This test article is not listed with the departmental database, and there is no obvious reason to presume that it is or contains 2,4-D. The volume is for US Borax products. The study is negative. This record appears to have been included by mistake, perhaps because a positive control agent was 2,4-dinitrophenol. No review. Aldous, 8/8/06.

142-0054 42297 This is information from an old formulation comprised of primarily "mixed aromatic solvents" plus small amounts of 2,4-D and PCP. It contains no reviewable chronic

data, and is unlikely to be of value to evaluation of contemporary 2,4-D products. Aldous, no worksheet, 8/8/06.
